Environmental Health Perspectives
Vol. 24, pp. 45–50, 1978

Developmental Aspects of the Male Reproductive System

by Jack Davies*

The development of the mammalian reproductive system involves: (1) an indifferent or ambisexual stage, in which both the male (Wolffian) and female (Mullerian) duct systems are present; (2) sexual differentiation, in which the phenotypic sex is expressed by the enhancement of Mullerian structures in the female and Wolffian structures in the male and reciprocal suppression of the opposite duct system; (3) cytodifferentiation, in which the epithelial, stromal and muscular features are regionally established; and (4) actual response to endogenous hormones, especially in mammals, such as the human and guinea pig, in which the differentiated tissues respond according to their capability. Specifically in the male, the onset of sexual differentiation is signaled by the elaboration of an androgenlike material (possibly testosterone) and a Mullerian-inhibiting factor from the testis. In the absence of these two influences, or one of them, the reproductive tract remains essentially female in configuration, a normal situation in the female and in abnormal males in which the urogenital sinus and Wolffian structures are incapable of hormonal responses due to the lack of specific enzymes or receptors. Male differentiation in particular involves enlargement of the penis and its canalization by the urethra, scrotal development and descent of the testis, and the formation of accessory glandular structures from the urogenital sinus or Wolffian ducts (bulbourethral gland, seminal vesicles, ampulla, prostate). Remnants of the Mullerian system may persist at the upper pole of the testis and are normally present (uterus masculinus) in relation to the prostatic part of the urethra.

The emergence of the characteristic parts of the male reproductive system in higher mammals in relation to phylogeny and the detailed origin of these in individual ontogeny are described. The use of the guinea pig as a model animal system for the study of transplacental effects of hormones (diethylstilbestrol, estradiol) and teratogens is briefly discussed.

Our interest in the development of the male reproductive system and the manner in which it responds to sex hormones was stimulated recently when we were approached by a leading drug house to develop an animal model for the study of diethylstilbestrol (DES) effects *in utero*. It seems appropriate to begin by reviewing for the benefit of the participants the evolution and development of the mammalian reproductive system in general terms. The reproductive system is one of the most conservative of the body systems, in common with the brain, in terms of evolution and ontogenetic development of the individual. The old paradigm, the biogenetic law that "ontogeny repeats phylogeny," is true to a striking degree in the mammalian reproductive system. In all mammals there are developed in craniocaudal sequence three renal systems of increasing complexity: the pronephros, the mesonephros (Wolffian body), and the metanephros (definitive mammalian kidney). The pronephros is rudimentary in all mammals; it is typical of free-living larval forms, such as the tadpole, in which there is no necessity for the conservation of water. It is characteristically segmental in origin, arising as outgrowth from the intermediate cell mass (nephrostome, segment stalk) which connects the somites to the lateral plate mesoderm. The outgrowths then grow caudally, linking up to form a longitudinal duct (archinephric duct) which opens into the cloaca or common chamber for the urinary and rectal passages. In mammals the pronephros, having given rise in this way to the duct, degenerates and leaves no traces. At a more caudal level (somite 10 and caudally in man) the segmental stalks from somites to lateral plate fuse into a linear cord of cells (the nephrogenic cord). The duct lies dorsal to it and is used by the next kidney to develop (mesonephros) as its conduit. The nephrogenic cord loses connec-

* Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee 37203.

tions with the coelomic lining and with the somites, and tubules are developed within it, in the case of the human embryo about three per segment. The ducts elongate, become S-shaped, and a glomerulus is developed medially by the formation of blood vessels *in situ*. Connections are established with the aorta (afferent arteriole) and the cardinal veins (efferent arterioles). The mesonephric tubule opens into the pre-existing duct, now the mesonephric or Wolffian duct, and a circulation is soon established, effecting a functional nephros capable of producing urine and modifying it in unknown ways. About 30 mesonephrons are formed in the human mesonephros (Wolffian body) in strict craniocaudal sequence. They begin to degenerate also in a craniocaudal manner before even the caudal nephrons are functionally developed. The fate of the mesonephros is crucial to the understanding of the male reproductive system, and will be resumed in the narrative after the origins of the Mullerian ducts and of the definitive kidney are summarized.

The Mullerian ducts arise relatively late in the human embryos (in embryos of about 10 mm and 5½ weeks) as thickenings of the coelomic (peritoneal) epithelium at the upper pole of the mesonephros. The plates sink in, forming a funnel, and then grow caudally first as a solid rod and then as a hollow tube. They enter the mesenchyme of the pelvis and fuse together, forming a solid "uterovaginal canal" which impinges on the posterior wall of the cloaca. The latter is subdivided into a ventral urogenital sinus and a dorsal rectum by the formation of a coronal partition or urogenital septum. When this is accomplished, the Wolffian ducts and the midline Mullerian ducts (fused) are found to open into the urogenital sinus. The metanephros or final kidney arises from the posterior surface of the Wolffian ducts immediately cranial to its point of entry into the urogenital sinus. It is arrested in its cranial growth by impinging on the lower end of the nephrogenic cord which forms a "metanephrogenic cap" around it and gives rise to the nephron or secretory units of the kidney; the ureteric outgrowth from the Wolffian duct becomes the ureter, the calyces and the collecting duct system.

Returning to the mesonephros, we next observe the origin of the gonad as a thickening of the coelomic lining anteriorly and medially to the upper end of the mesonephros. It is in close contact with the glomerular capsules of the mesonephric tubules from about the 7th to the 11th somite (human embryo). Felix, in his classical account of the human reproductive system (*1*) describes three parts of the mesonephros (see Fig. 1). They are a presexual portion (nephrons 1 to 6), a sexual portion opposite

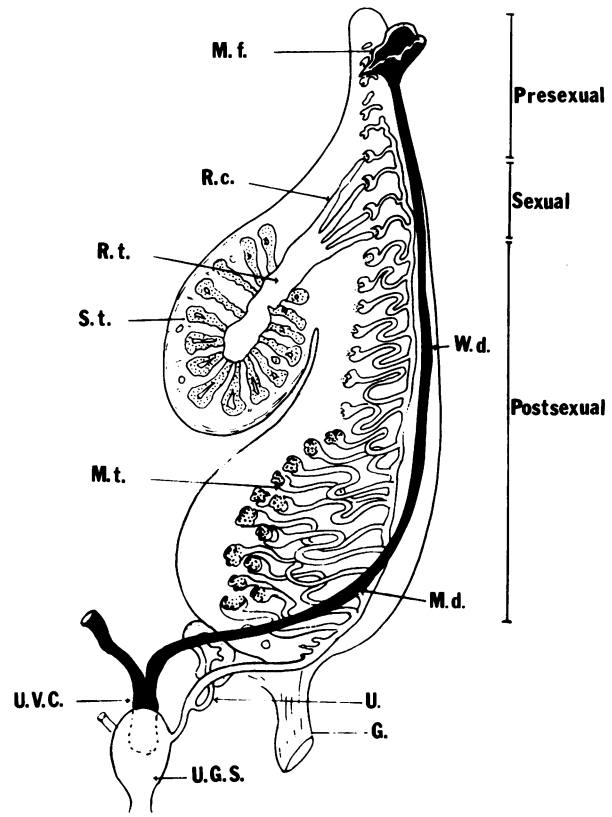

FIGURE 1. Diagram of the developmental parts of the mammalian reproductive system: (G) gubernaculum; (M.d.) Mullerian duct; (M.f.) Mullerian funnel; (M.t.) mesonephric tubules and glomeruli; (R.C.) rete cords, (R.t.) rete testis; (S.t.) seminiferous tubules; (U) ureter; (U.V.C.) uterovaginal canal; (U.G.S.) urogenital sinus, (W.d.) Wolffian duct.

the gonad (nephrons 7 to 11), and a postsexual portion, posterior to the gonad. The presexual portion degenerates with few traces, occasionally giving rise to cystic remnants (appendix testis in the human). The postsexual portion continues to function as the important kidney of the fetus until about the end of the third month after which it degenerates leaving a few traces (paradidymis in the male, paroophoron in the female). The metanephros or definitive kidney overlaps the mesonephros functionally during the third month.

The sexual portion of the mesonephros, so far from being rejected, is transformed into the conduits of the gonad by a remarkable process of "urogenital union." Felix has described this process in great detail in the human embryo. The gonad in both sexes, as are the Wolffian and Mullerian ducts, are common to both sexes ("ambivalent stage"), lasting to about the 8th week in the human. During this time the sex cells (oogonia in the female, spermatogonia in the male) enter the primitive gonad

from an extragonadal source. The sex cells appear to arise in the region of the cloaca, perhaps from the endoderm in this area. They are large vesicular cells, give a characteristic periodic acid Schiff reaction (indicating polysaccharides) and also a positive alkaline phosphatase reaction. Blandau (*2*) has also shown by time-lapse cinematography that they are ameboid. They presumably make their way into the dorsal mesenteries of the gut and enter the gonad through the attachments or mesenteries uniting it to the posterior abdominal wall and to the gonad (mesorchium in the male, mesovarium in the female). This primary set of germ cells, which are diploid like the somatic cells, exert an inductive influence on the urogenital ridge, which contains the mesonephros and the Wolffian and Mullerian duct system. Genetically, male germ cells are able to induce the urogenital structures to differentiate into male structures, and vice versa for the female, regardless of the genetic make-up of the urogenital ridge itself. The gonad first indicated phenotypically its sexual significance by the appearance of several parts. In the female the gonad forms a central core or rete and a peripheral cortex in which the oogonia are encapsulated by mesenchymal cells, forming primordial follicles. According to current theory, a second proliferation of coelomic cells is added in the female to the existing gonad which now becomes the medulla (essentially male), and the new cortical component, not represented in the female, is the essentially female component of the ovary. In the male the accession of new proliferation of cells from the surface coelomic epithelium is prevented by the formation of a connective tissue layer beneath the peritoneal surface, the tunica albuginea. Henceforth the male gonad is represented only by the first or medullary component (male). The theory, developed in part by Witschi (*3*) in amphibians, and also probably valid for the birds, accounts for the tendency of females to turn into males, e.g. the hen into a rooster. The female gonad is potentially bisexual according to this view, and the testis does not possess this double potentiality. In the mammals the theory is much less easy to establish on a morphological basis but continues to be the basis for speculation on a bisexuality in human patients.

The urogenital union is accomplished in both sexes by the fusion of the rete with the glomerular capsules from the 7th to about the 11th. Solid cords (the cords of Mihalkovics) are the medium of this connection. These appear to infiltrate the glomeruli which are later extruded from Bowman's capsule (glomerular decapsulation), leaving the cords in organic continuity with the tubules from about the 7th to the 11th. Later the rete cords canalize as do the rete and the seminiferous tubules within the cortex of the gonad. The mesonephric tubules become the efferent ductules of the adult testis. The mesonephric or Wolffian duct becomes differentiated with much elongation as the duct of the epididymis and, more distally, the vas deferens. The parts of the adult testis and epididymis and their embryological basis are illustrated in Figure 2.

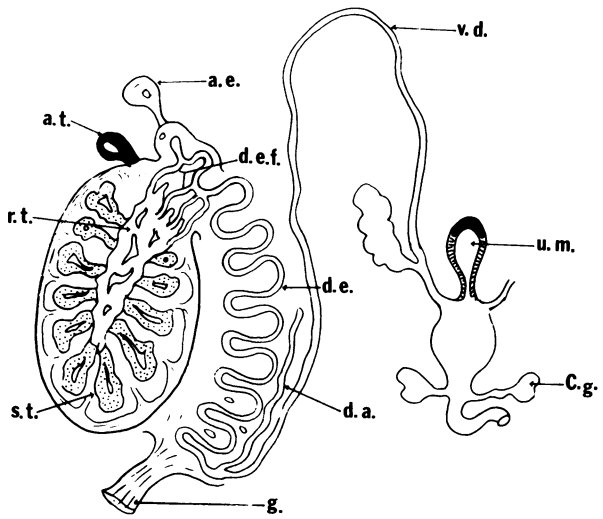

FIGURE 2. Diagram of the adult male derivatives of the embryonic bisexual structures (see Fig. 1): (a.e.) appendix epididymidis; (a.t.) appendix testis; (C.g.) Cowper's gland; (d.a.) ductus aberrans of Haller (remnant of postsexual part); (d.e.) ductus epididymidis; (d.eff.) ductuli efferentes (from sexual part); (g.) gubernaculum (inferior scrotal ligament); (r.t.) rete testis; (s.t) seminiferous tubules; (u.m.) uterus and vagina masculin(us)a; (v.d.) vas deferens (from Wolffian duct).

In the female the urogenital union is abortive. The rete cords degenerate except for remnants in the hilus of the ovary which may become cystic. The efferent ductules of the male are represented by the epoophoron, a system of regressed tubules in the broad ligament. The Wolffian duct may persist as the vestigial duct of Gartner; it opens, when persistent, into the vestibule of the vagina near the base of the hymen. It also may give rise to cysts.

The subsequent phenotypic expression of genetic sex in the embryo and fetus is dependent on the inductive effect of the gonad. This is true only in the male in which the testis elaborates two principles or morphogenetic substances, a testosteronelike material and a Mullerian-inhibiting factor (MIF). The former is mimicked by testosterone and may actually be testosterone, inasmuch as the Leydig cells are differentiated about the time of onset of sexual dimorphism. The MIF is probably polypeptide in nature. The effect of these two substances is to

stimulate the urogenital sinus and associated structures to differentiate in the male direction (prostate, Cowper's gland, seminal vesicle, ductal differentiation of vas and stromal vessels and, in the case of MIF, to cause the involution of the Mullerian ducts. In the female, the gonad apparently exerts no morphogenetic influence, so that in the absence of a positive stimulus to male development the embryonic and fetal reproductive structures remain female by default. In the female the Mullerian ducts normally enlarge and become the upper vagina, cervix, uterus and uterine tubes, and the Wolffian structures regress. In view of these facts, it is reasonable to understand how male hormones may masculinize a female fetus, as in the administration of progesterone (a weak androgen) in pregnancy, but impossible to feminize a male. These facts are significant in considering the theoretical effect of a nonsteroidal material such as diethylstilbestrol on the female fetus. Feminization would not be expected.

In evolutionary terms the development of the human reproductive system reflects the essential events. In primitive vertebrates, e.g., shark, the eggs are shed from the ovary into the peritoneal cavity and conveyed to the outside through pores near the cloaca. Fertilization is in water and outside the shark's body. The Mullerian duct system appears to be developed phylogenetically, as it is in the ontogeny of mammals, as a groove in the coelomic or peritoneal lining. This closes to become a tube but remains open cranially to receive the eggs shed from the ovary, forming the ostium abdominale or fimbriated extremity of the uterine tubes. The Mullerian ducts fuse in true (eutherian) mammals to form a uterus and vagina with or without urogenital sinus contributions to the distal vagina. The germ cells probably arise in all vertebrates from an extragonadal site, and may be fundamentally endodermal in origin.

The division of the cloaca first appears in the monotremes (protherian mammals, e.g., duck-billed platypus). The metatherian mammals (e.g., marsupials) present a fundamental divergence from the line which gave rise to the eutherian or true mammals in that the Mullerian ducts remained unfused, hence the name Didelphydae or "double-uterus mammals." Accessory glands appear in the marsupials as a clustering of glands around the urethra and bladder neck. A diffuse prostate with no seminal vesicles, and periurethral glands including Cowper's glands, appear in the monotremes. The division of the cloaca is complete in the true mammals, and it is from the anterior or urogenital sinus component that the prostate emerges. Rodents are characterized by complex prostatic structures, e.g., ventral, dorsal and lateral complexes, and the coagulating gland dorsally which is concerned with the formation of the copulatory plug. The prostatic complex in primates and in man is characteristically compact, though subdivisions may be recognized within it. The copulatory organ itself, the penis, shows infinite complexity of structure and development in all vertebrate forms. Diversity of structure and plasticity of development are most marked in the external genitalia; the internal genitalia, notably those derived from the Wolffian and Mullerian ducts, are conservatively preserved in their essentials. It seems axiomatic that old and conservative systems are less susceptible to developmental abnormalities, natural or induced, than are the more recent plastic structures. In like fashion, the ancient components of the skull, the base of the skull, and the special sense capsules, are rarely involved in congenital abnormalities; conversely the phylogenetically recent parts of the skull, the membrane or dorsal bones of the facial skeleton and the cranial vault are more susceptible.

Our original contribution to this symposium is limited to a few comments about the use of the guinea pig as a model animal system for the study of the intrauterine effects of nonsteroidal hormones. The clinical problem in women is one of some poorly understood disturbance in genital tract development in the offspring of women who received diethylstilbestrol during pregnancy. The lesion in females is the presence of columnar epithelium and glands of cervical or endometrial type within the upper vagina (adenosis), and a tendency in some girls to develop clear cell adenocarcinoma of the vagina or cervix in the prepubertal or early adult years, a very rare circumstance under normal conditions. Exposure to DES, especially in the first trimester of pregnancy is established in most cases, though a causal relationship to the malignant lesion is not established. The critical period is obviously one related to the period of sexual differentiation following the ambisexual stage (after the 8th week) and the prolonged period of cytodifferentiation and regional specialization of the genital tract that follows. Exposure of newborn mice to natural estrogen (*4*), e.g., 1 μg of estradiol/day for 5 days, causes persistent vaginlinaal cornification. The animals fail to cycle in later life, and a condition said to mimic adenosis in women is said to exist in older animals, and in some cases tumors have developed in susceptible strains. It is important that in most experimentally used rodents (mice, rats, rabbits) the cytodifferenation of the vagina, uterus, and tubes takes place for the most part after birth. In the human fetus it is begun during the third month and continues progressively to term. Moreover, in the

human fetus, the genital tract epithelia and stromal structures acquire the capacity to respond to endogenous sex hormones, notably estrogen, and do so beginning about the 20th week (5). At birth the female child shows marked stimulation of the genital tract, probably estrogenic, and after birth there is a rapid regression of these intrauterine effects, called by Courrier (6) "la crise génitale du nouveau-né." We have chosen the guinea pig as our experimental animal, since it resembles more closely the human situation during pregnancy than do the common laboratory animals. Thus the gestation period is relatively long (about 65 days), the fetus is well developed at birth, the cytodifferentiation of the genital tract takes place almost entirely during fetal life, the genital tract shows responses to endogenous sex hormones, probably estrogens but perhaps also progesterone, and the embryology and adult structure of the vagina, cervix, and related parts are similar to those of women, allowing for some histological differences.

We are not in a position here to report on the effects of intrauterine exposure to DES or natural estrogens. We shall comment briefly on what has been established concerning normal male and female development in the guinea pig, using this as a paradigm of all mammalian genital development. The ambisexual phase, in which both sex ducts are present and genetic sex is not expressed, was found to be from about 20 to 28 days. The pattern of development is very similar to that of the human though more rapid. One major difference is that the mesonephros is less advanced at any stage than in the human embryo and remains smaller and less complex. By day 28 sexual differentiation is underway. The Mullerian ducts are fully formed and make contact with the urogenital sinus. Between 33 and 35 days the primordium of the vagina (and vagina masculina in the male) appears as a proliferation of the epithelium (endoderm) in the posterior wall of the urogenital sinus. The epithelium of the sinus is characteristic, consisting of stratified cells with vacuolated cytoplasm, the vacuoles being related to their *in vivo* content of glycogen which is dissolved out in preparation. The human vagina (7) develops in a similar way. The sinus proliferation impinges on the fused Mullerian ducts, causing them to be displaced cranially. Ultimately the vagina is formed from the sinus proliferation and the fused Mullerian ducts. We have established that most of the vagina is Mullerian in the guinea pig and that only the distal part, notably the so-called vaginal plug or "membrane" is of urogenital origin. The human vagina has been considered historically to be either of Mullerian origin, urogenital sinus origin, or a mixture of both. The epithelium of the Mullerian vagina is quite different from that of the urogenital sinus portion, being initially a pseudostratified columnar one lacking glycogen. Urogenital sinus derivatives are invariably stratified and rich in glycogen, at least in the early stages.

Cytodifferentiation of the Mullerian vagina begins about 33 to 35 days in an ascending or caudocranial manner, a pattern also observed in the mouse, and probably in all mammals including the human. Involution of the Mullerian ducts is advanced at the same stage in the male and six prostatic buds or outgrowths are apparent from the urogenital sinus on either side. The cavernous bodies of the clitoris and penis also apear. The seminal vesicles are present as an outgrowth of the distal end of the vas deferens. Between 35 and 40 days the proliferation of the prostate continues, and in the female cytodifferentiation of the vagina has extended to about half way up the Mullerian vagina. The Mullerian ducts are lost in the proximal and intermediate parts in the male and remain as bifid rudiments on top of the sinus proliferation (vagina masculina and uterus masculinus). The cervix is differentiated in the female about the 43rd day. Evidences of Leydig cell activity are present in the testis. By the 50th day in the female the vagina is transformed up to the cervix and shows early signs of endogenous hormone (estrogenic?) stimulation. Cytological differentiation of the prostatic complex, Cowper's gland, and the lining epithelia and muscular components of the vasa deferentia and seminal vesicles is well advanced but without evidences of actual secretion. At birth (about 65 days) stimulation of the genital structures in the male and the female is evident. In the female the vagina and cervix are superficially mucified. In the male there is hyperplasia and some superficial mucification of the common chamber into which the vasa deferentia, the seminal vesicles, and the vagina masculina open, and also of the distal part of the vagina masculina. Postnatally there is no apparent decline in the stimulatory effects observed at birth, in either sex. In the male secretory activity in the prostate and accessory glands is apparent by the second week and continues without abatement until puberty at about three months. The analogy with the human fetus is greater than in the case of any common laboratory animal, and we consider that it will serve as a useful model system for the study of DES-effects *in utero*.

In particular, based on the normal table of development outlined above, we hope to observe effects of DES, also of estradiol and progesterone, on the embryonic and fetal genital tract in both the male and the female. The hormonally sensitive tissues are clearly those derived from the urogenital sinus and those from the Mullerian duct system. In

the male these areas are the prostatic area in general and the remnants of the Mullerian system (vagina masculina and uterus masculinus). The reacting tissues may be expected to respond in a manner typical of their origin and epithelial characteristics. In view of recent observations (*8*) that effects the DES exposure *in utero* are also to be observed in males (hypospadias, penile urethral atresia, cysts of the epididymis, gonadal hypoplasia, impaired fertility, etc.), the use of the male fetal guinea pig as a model system is timely. Long-term studies will also be carried out to determine to what extent the DES-induced effects before birth are prolonged into postnatal or adult life, and most especially with reference to the development of malignant lesions.

This work was supported by a grant from Eli Lilly and Co., Inc., Indianapolis, Indiana.

REFERENCES

1. Felix, W. The development of the urogenital system. In: Keibel and Mall's Human Embryology, Vol. 2. Lippincott, Philadelphia, 1912.
2. Blandau, R. Demonstration. Meeting of the American Association of Anatomists.
3. Witschi, E. Modification of the development of sex in lower vertebrates and in mammals. In: Sex and Internal Secretions. 2nd ed. Allan, Danforth, and Doisy, Eds., Williams and Wilkins Co., Baltimore, 1939.
4. Forsberg, J. G. Late effects in the vaginal and cervical epithelia after injection of diethylstilbestrol into neonatal mice. Amer. J. Obstet. Gynecol. 121: 101 (1975).
5. Davies, J., and Kusama, H. Developmental aspects of the human cervix. Ann. N. Y. Acad. Sci. 97: 554 (1962).
6. Courrier, R. Endocrinologie de la Gestation. Masson et Cie, Paris, 1945.
7. Bulmer, D. The development of the human vagina. J. Anat. 91: 490 (1957).
8. Bibbo, M., et al. Cytologic findings in female and male offspring of DES-treated mothers. Acta Cytol. 19: 568 (1975).